FIG. 1

Receptor Assay (1)

R —[L]— D   +   [L]—D

R —⊏

$G_{(T)}$

L_{free}

$\frac{1}{N}$

L_{bound}

log t

+ potential active substance

R —⊏

2x [L]—D

R —⊏

R —[L]— D

R —[L]— D

- antagonistic activator
- antagonistic blocker
- allosteric blocker

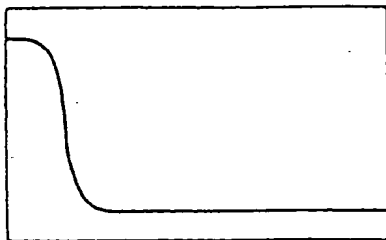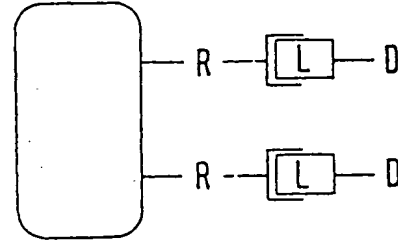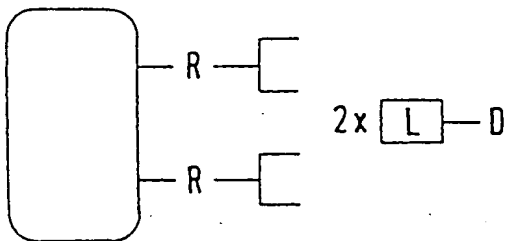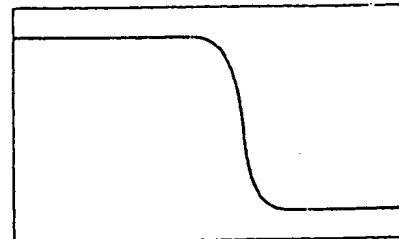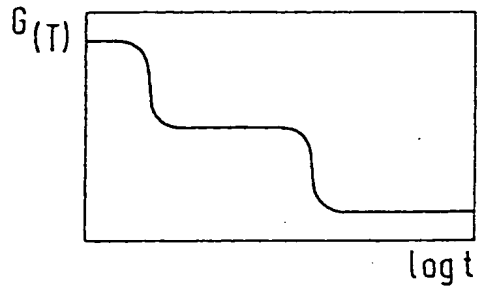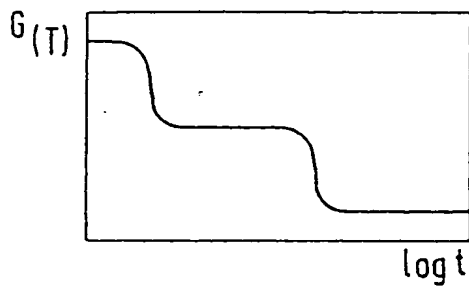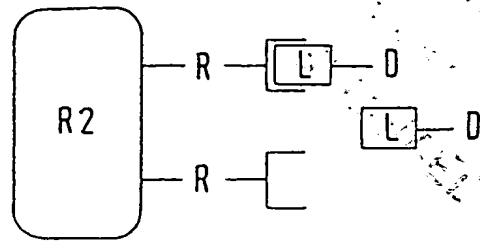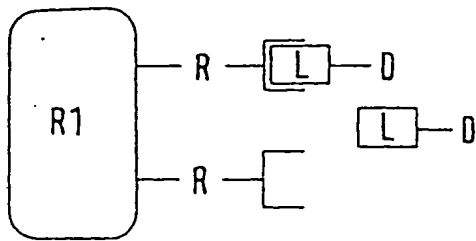- allosteric complex stabilizer
  (blocker or activator)

FIG. 2

Receptor Assay (2)

+ potential active substance

separation of
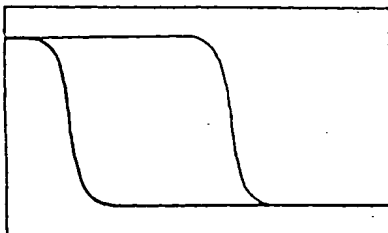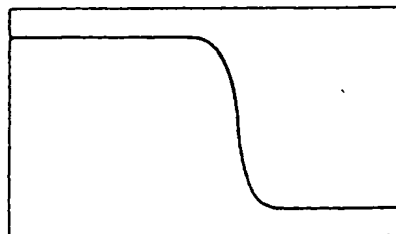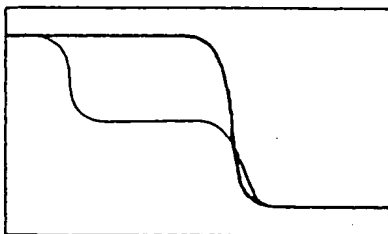receptor functions
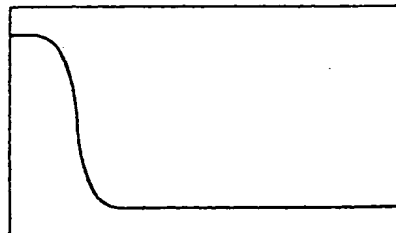
interference acting
in the same direction
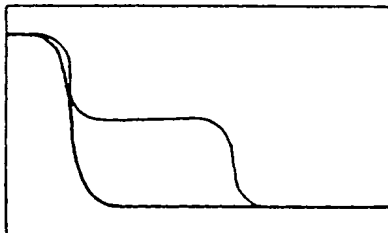
FIG. 3

FCS Analysis with Multi Well Sheets

multi well
carrier sheets

sample

optics

H_2O

Laser

Multiplier / Correlator
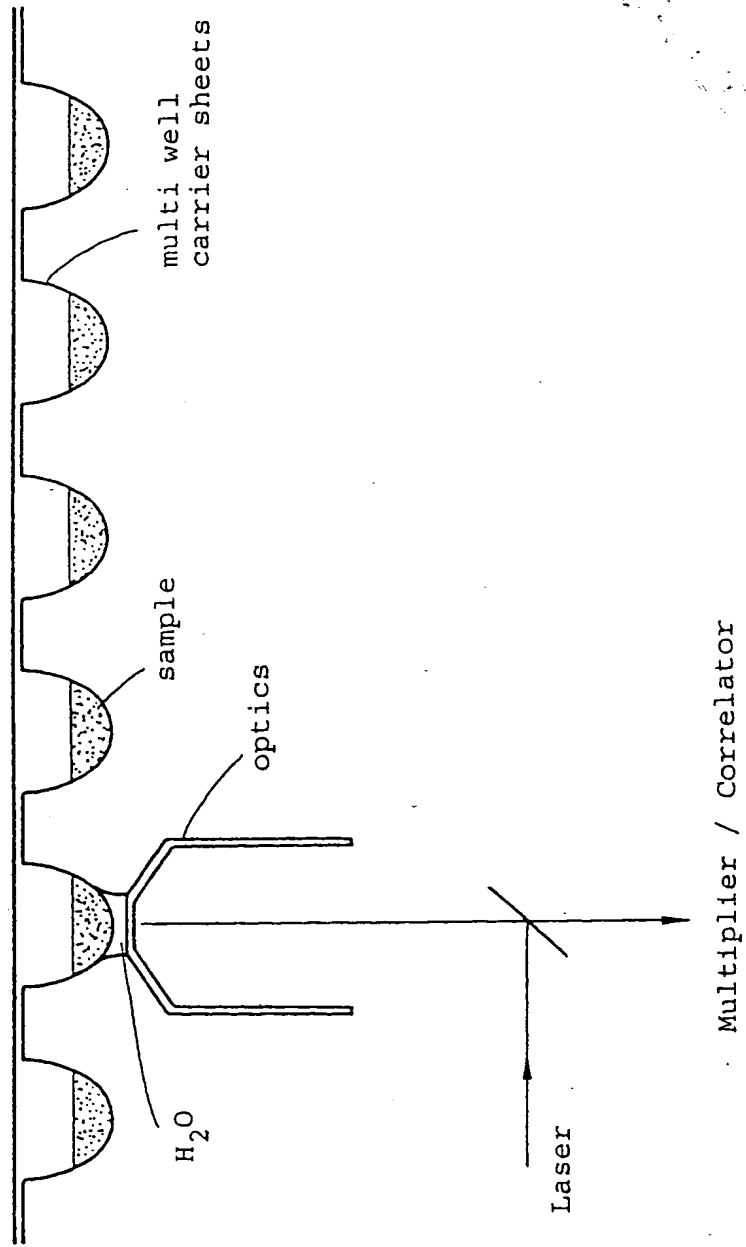
FIG. 4

FCS - Determination of the Fitness of Mutants

- Molecular Detection
- Concentration
- Equilibrium Constant
- Rate Constant
- Product Determination
- Mobility Assay
- Conformational Change

emitted
fluorescence

Correlator

Laser beam

10^4-10^6 Samples
per cm^2

y

x

Tube

- Sheet
- Capillary Wall
- Wafer
- etc.

cross-sectional view

H_2O

100-$1000\mu m$

10^{-14}-10^{-16} l
volume element

Aqueous sample with
(non-) addressed
position
- Droplet
- Cell
- Vesicle
- Gel Pore
- Capillary Segment
- AFFYMAX Element
- etc.

Chemical Coating
- Metal Chelate
- Ion Exchanger
- Photoreactive Ligand

Protecting Matrix
- Gas Phase
- Hydrophobic Liquid
- Lipid Coat
- Polymeric Matrix

FIG. 5
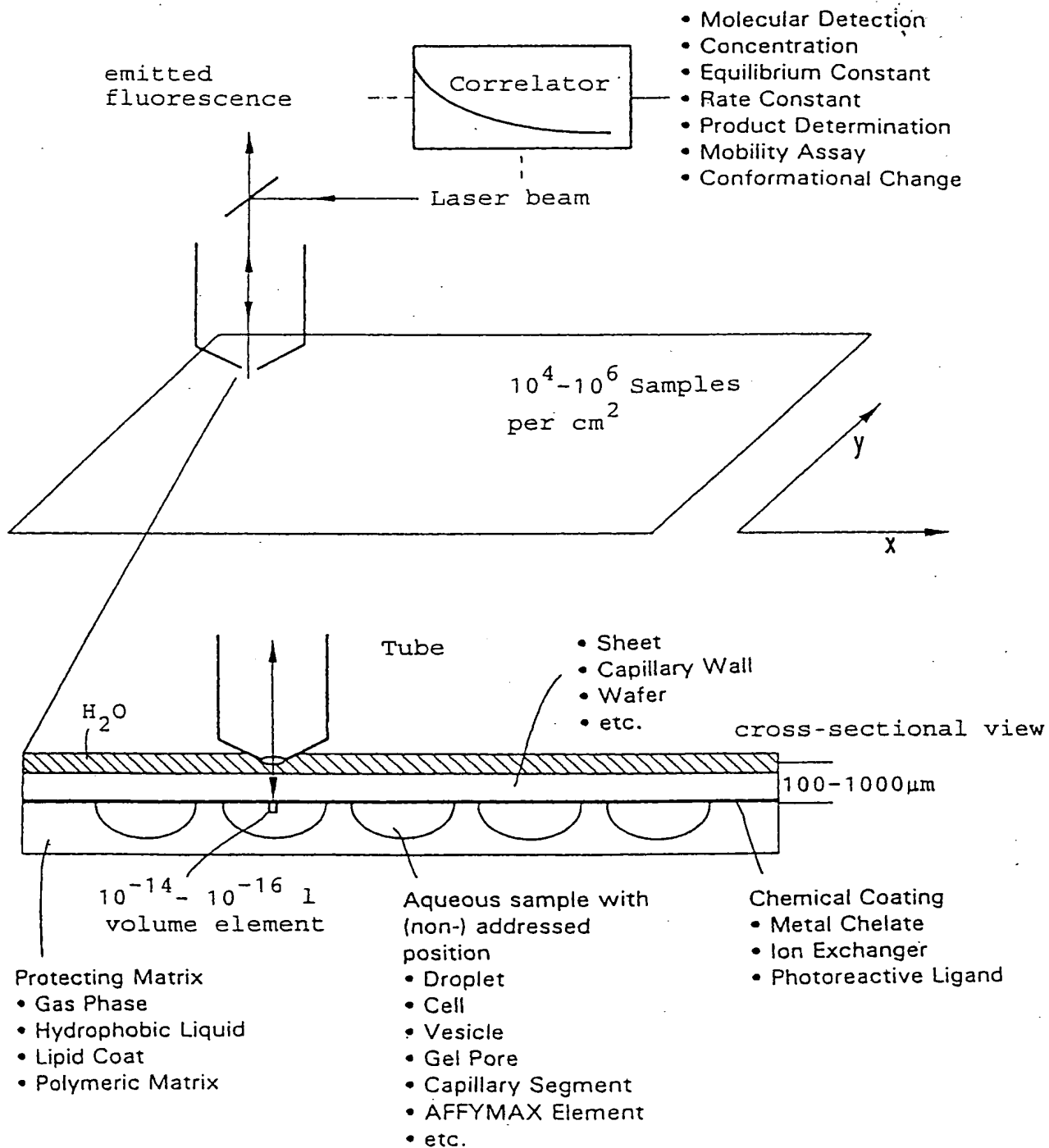
Detection of Molecules on
stationary structures through
relative temporal change of the
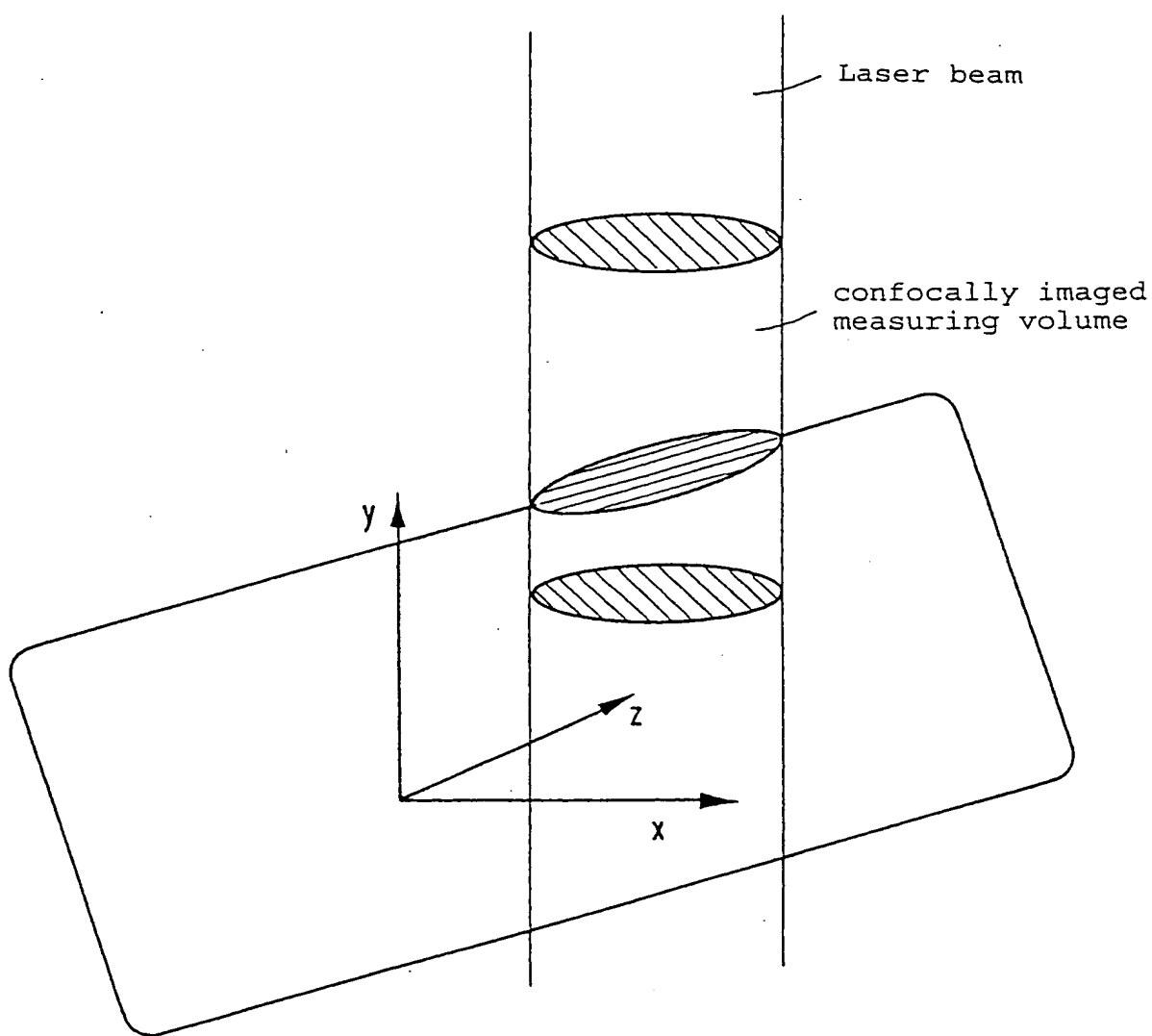positional coordinates of the
measuring volume

Laser beam

confocally imaged
measuring volume

FIG. 6

Detection of Single Molecules
in the Electric Trap

Detector 2

Laser

confocal optics
with high aperture

−   +

Microcapillaries with
chromium/gold vapor deposited
coating on the outside fix
the sample volume with
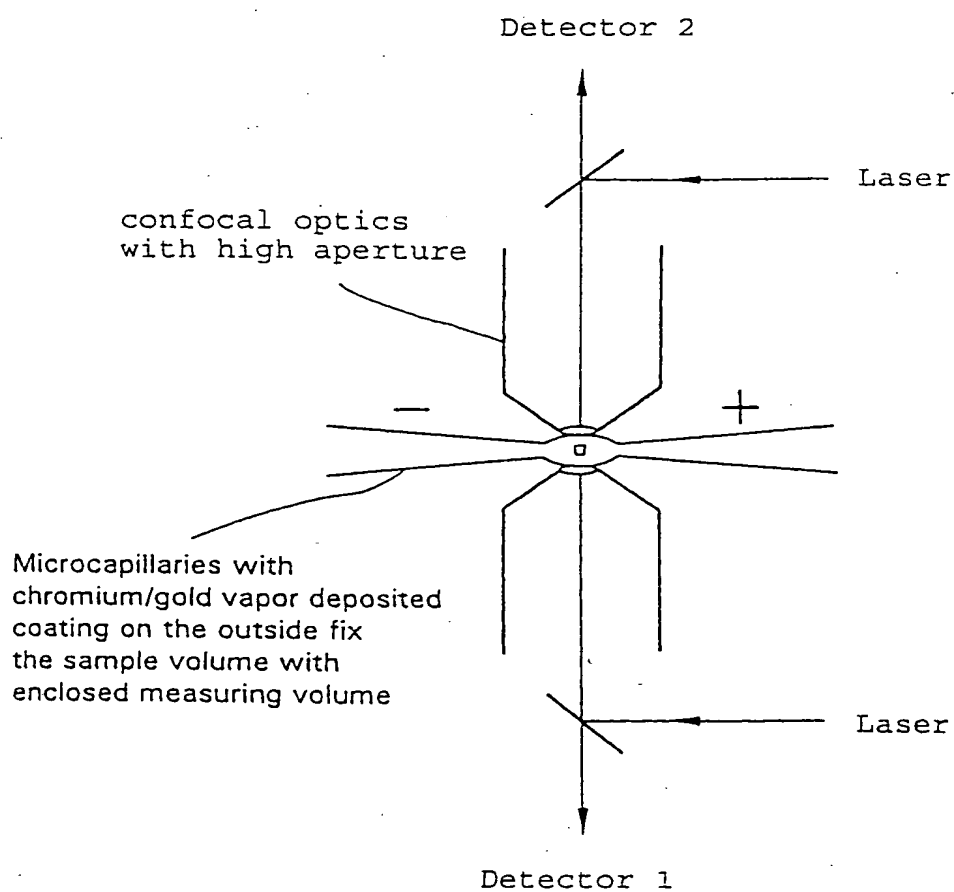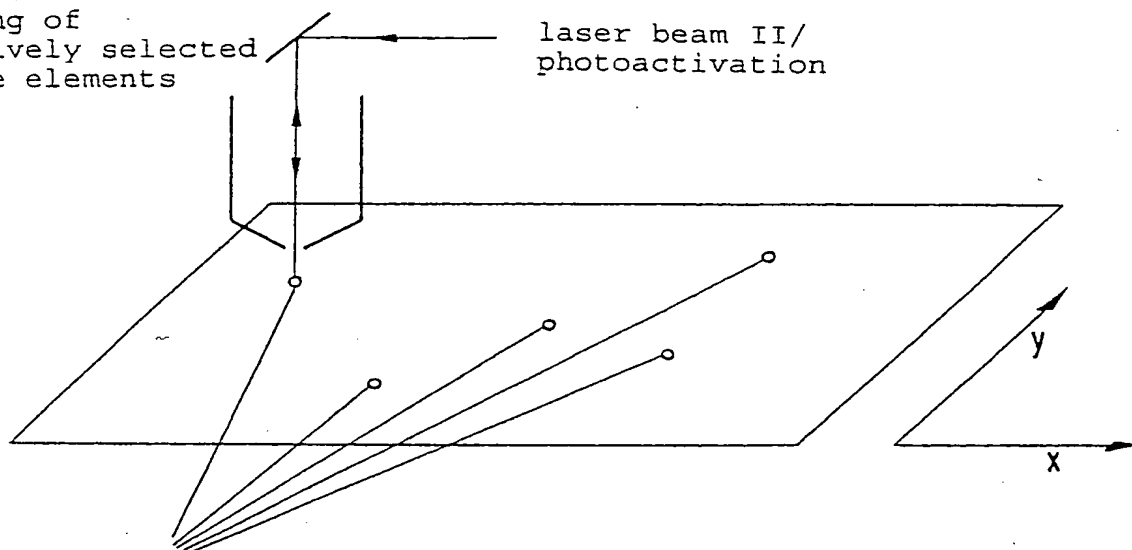enclosed measuring volume

Laser

Detector 1

FIG. 7

FCS - Tagging of the
Selected Genotypes

Photoactivated
tagging of
positively selected
volume elements
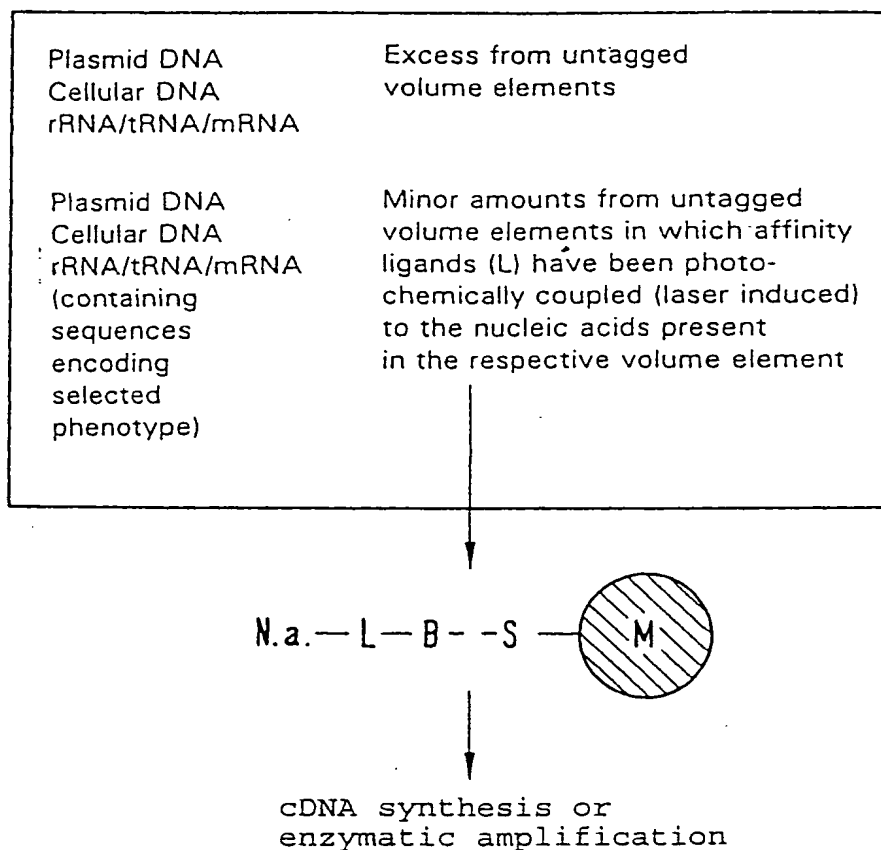
laser beam II/
photoactivation

y

x

a) Physical access to optically
tagged volume elements

b) Light induced linking of the nucleic acid
of selected volume elements to affinity ligands
- at the carrier surface
- to soluble ligands

FIG. 8

Preparation of the DNA/RNA of
FCS Selected Genotypes

Mixture of all nucleic acids
after phenotype evaluation:

Plasmid DNA Cellular DNA rRNA/tRNA/mRNA	Excess from untagged volume elements
Plasmid DNA Cellular DNA rRNA/tRNA/mRNA (containing sequences encoding selected phenotype)	Minor amounts from untagged volume elements in which affinity ligands (L) have been photo- chemically coupled (laser induced) to the nucleic acids present in the respective volume element

$$N.a. - L - B - -S - \underset{\displaystyle M}{\bigcirc}$$

cDNA synthesis or
enzymatic amplification

N.a.; Nucleic acid.
L; Ligand with specific nucleic acid affinity which can be photochemically coupled
covalently and preferably reversibly to a nucleic acid (e.g. a psoralen derivative).
The ligand is preferably linked to a substituent which allows for subsequent enrichment
of the nucleic acids. For instance, this can be a hydrophobic substituent to purify
nucleic acids by reversed phase chromatography. For affinity chromatography,
substituents such as biotin (B) are the obvious suitable ones so that the nucleic acids
can be enriched through (strept)avidin complexing (S) with appropriately modified
magnetobeads (M) or surfaces.

FIG. 9

FLUCS Analysis of Complex Mixtures
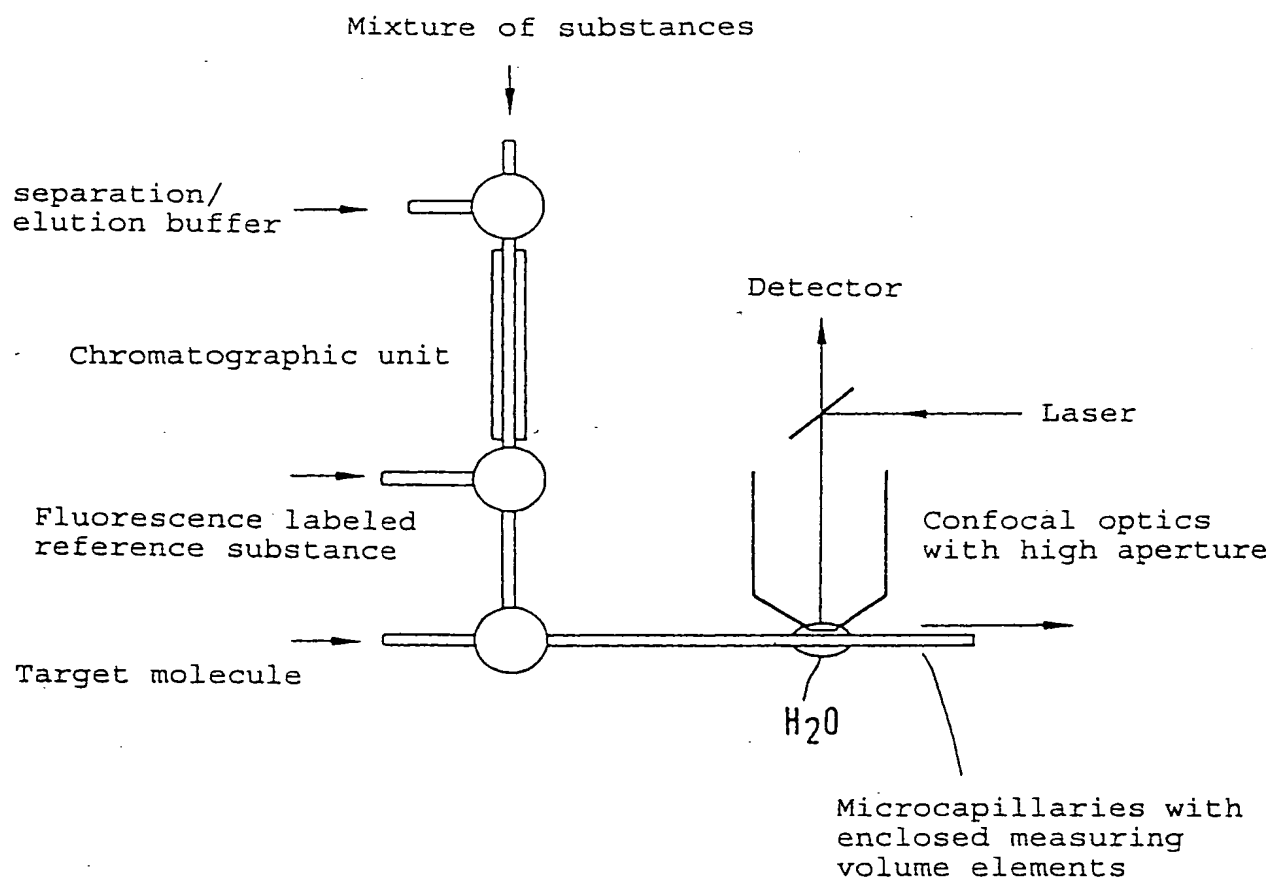of Substances after Chromatographic
Separation in Fractions

Mixture of substances

separation/
elution buffer →

Chromatographic unit

Detector

Laser

Fluorescence labeled
reference substance

Confocal optics
with high aperture

Target molecule

H_2O

Microcapillaries with
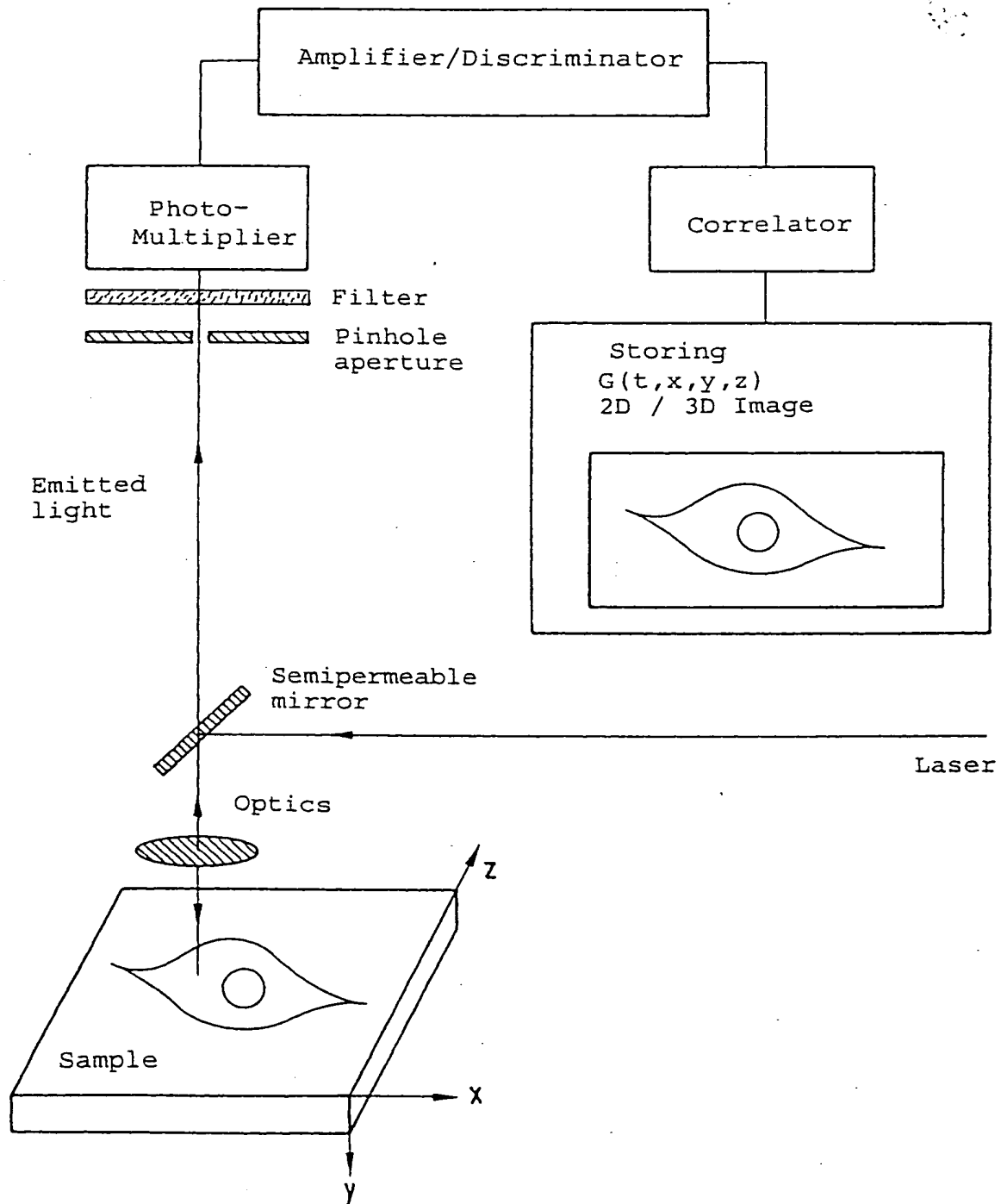enclosed measuring
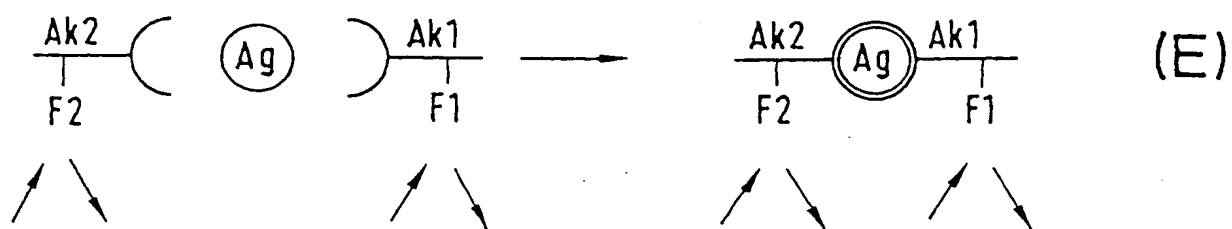volume elements

FIG. 10

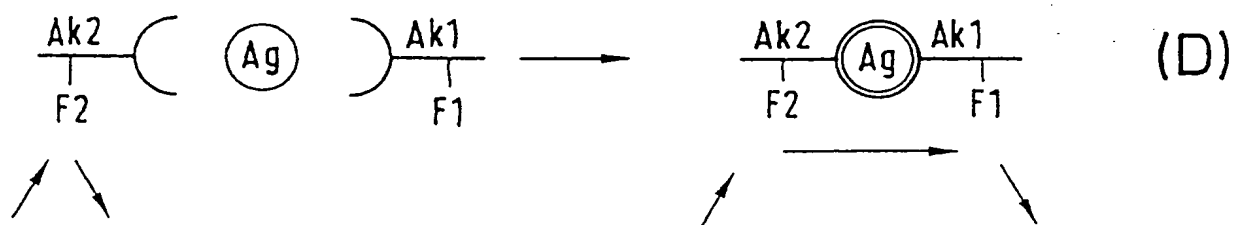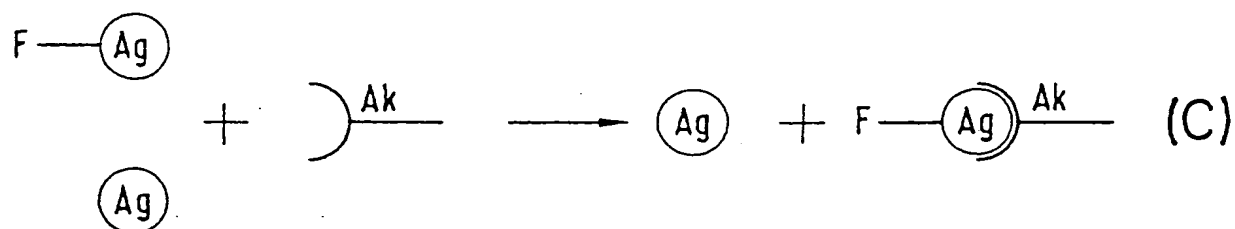Laser Correlation Microscope

FIG. 11

Selection of Possible Assays
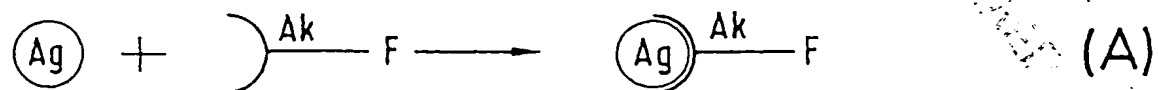
FIG. 12

Electrophoresis Cell

FIG. 13

Laser

Upper sextupole
(rectified field)   U = -100 V

Quadrupole   U = 0 V
(alternating field)

Lower sextupole
(rectified field)   U = -100 V

FIG. 14

FIG. 15

B_2 B_1

30

24

25

23

L

21 20

FIG. 16

53

58

56

60

55

50

59

57

52

FIG. 17a

channel time

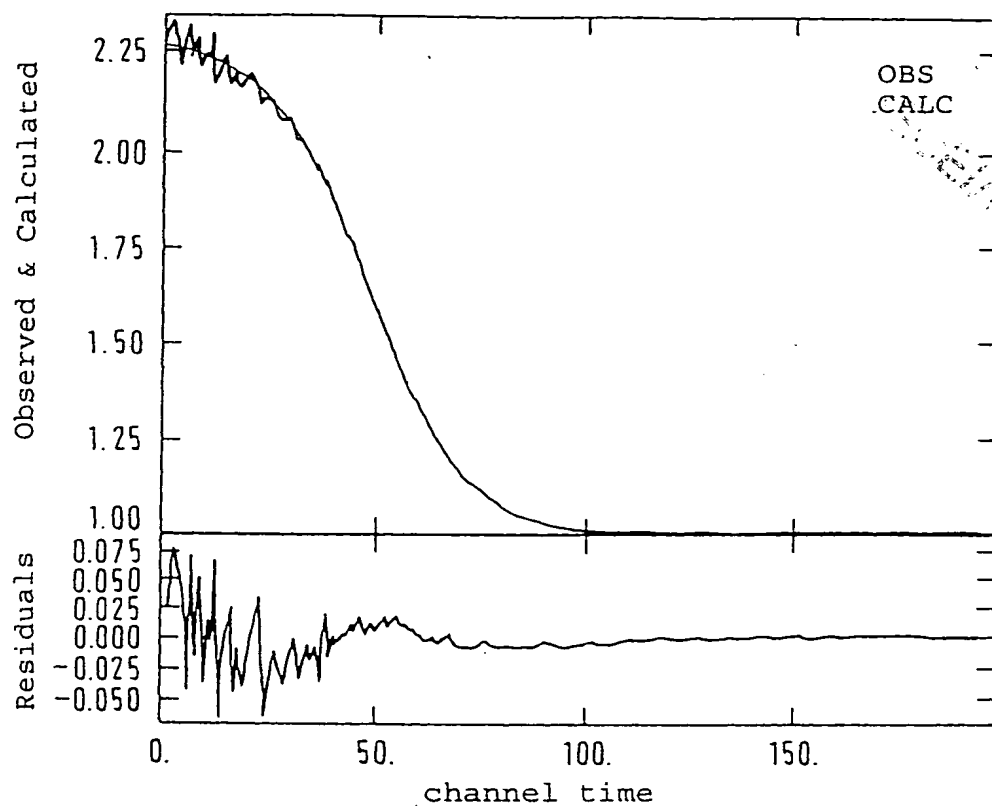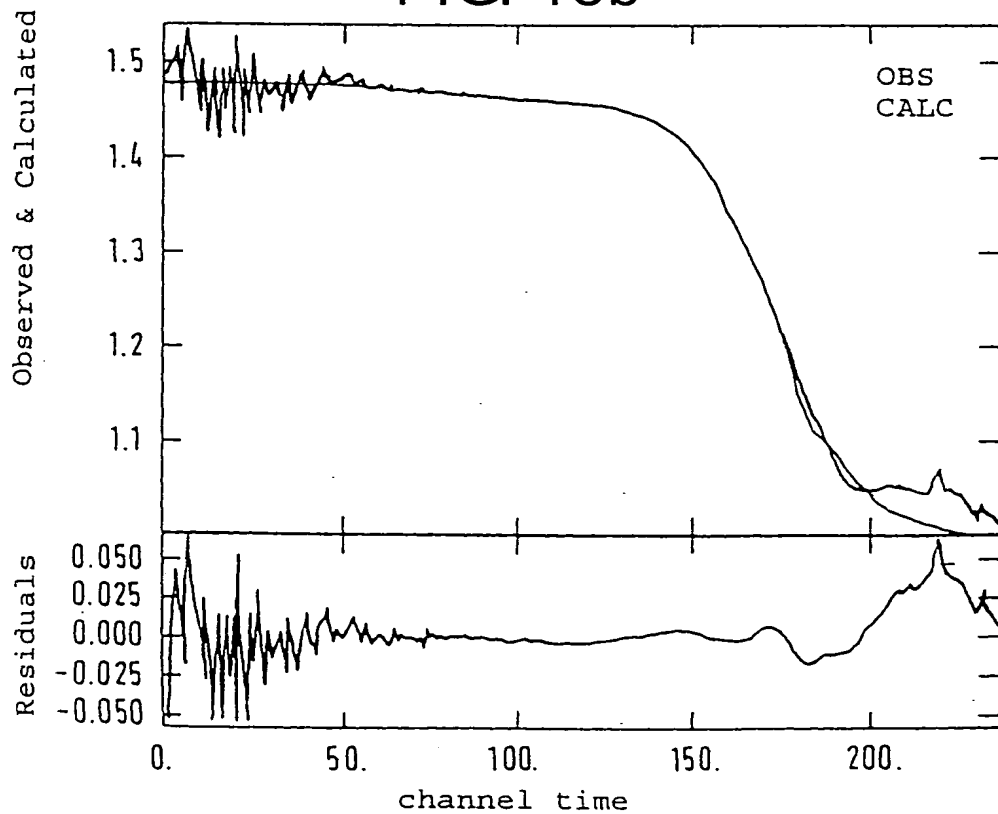# FIG. 17b

channel time
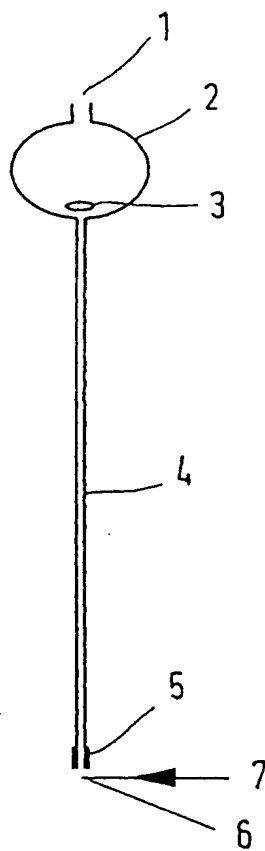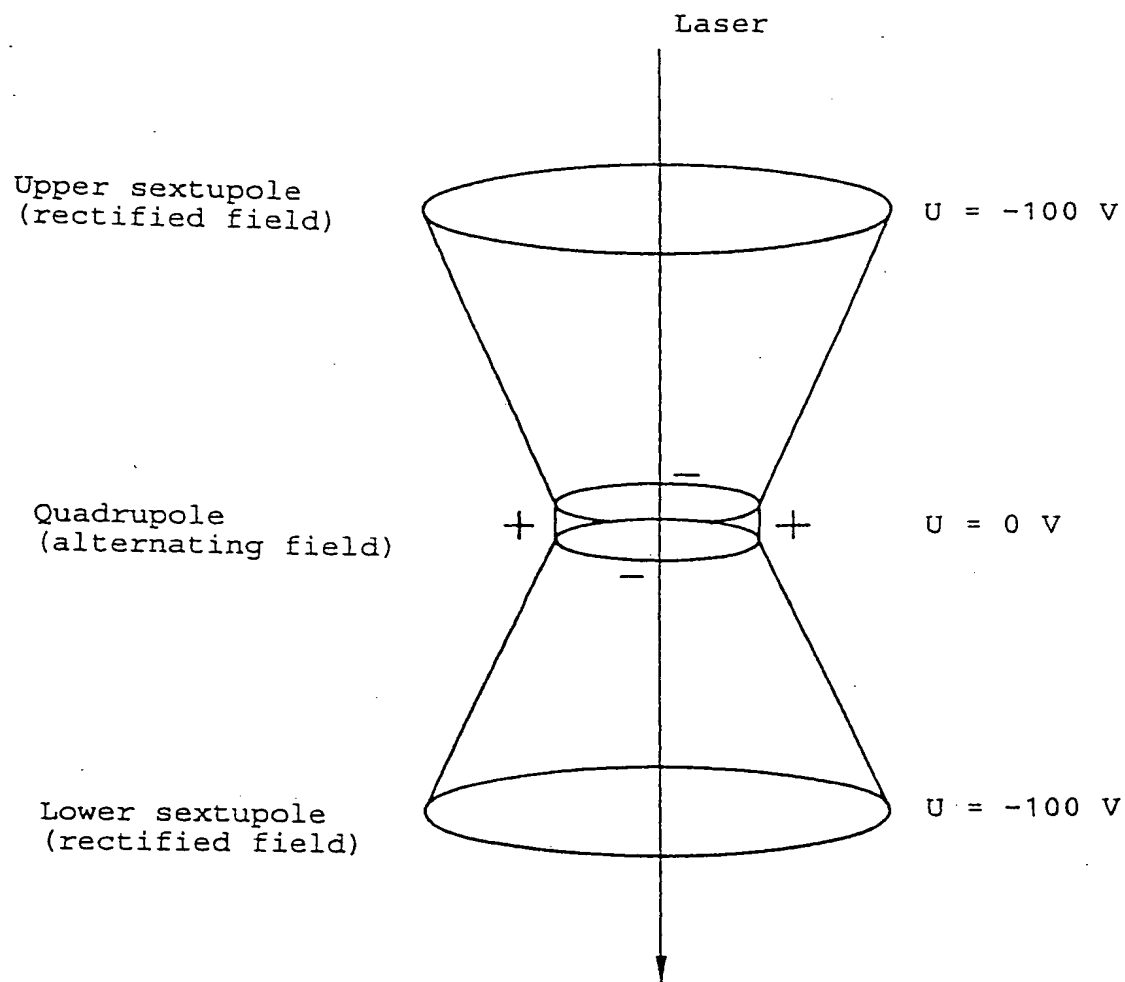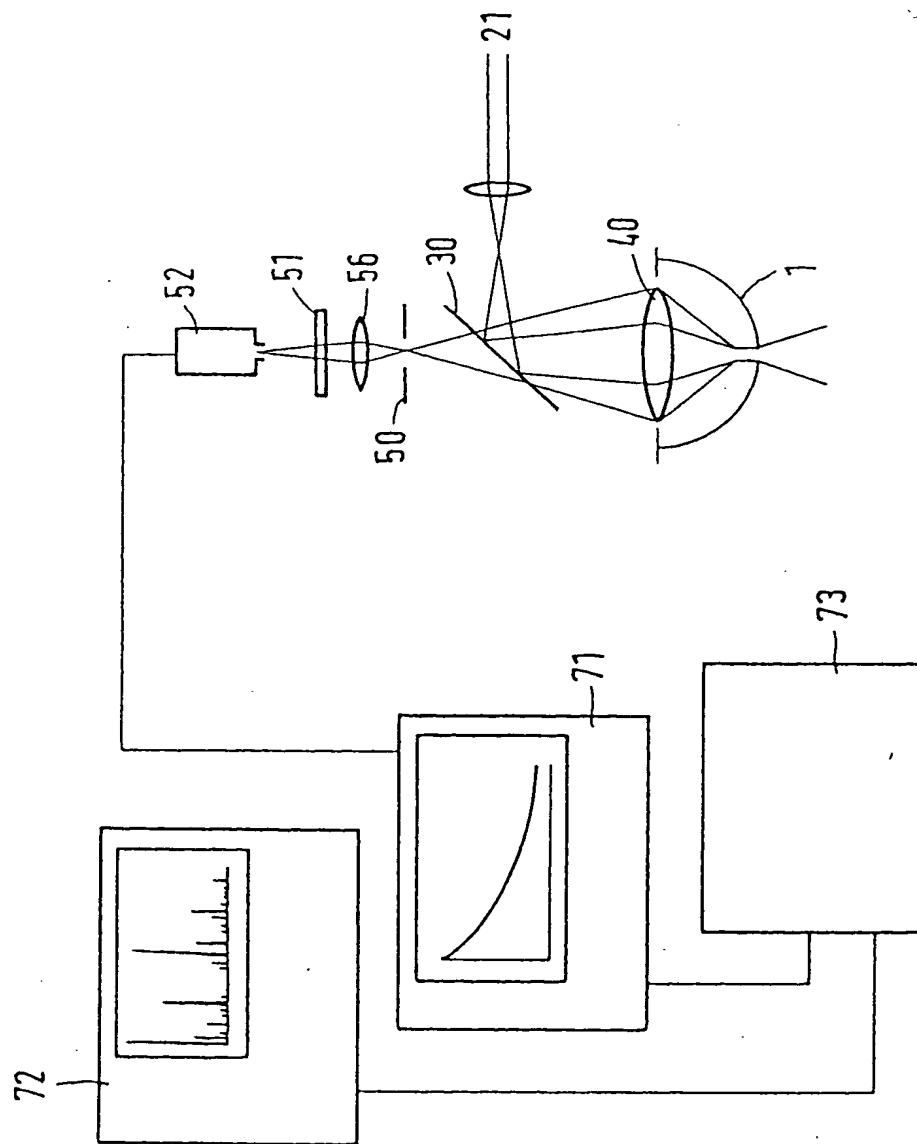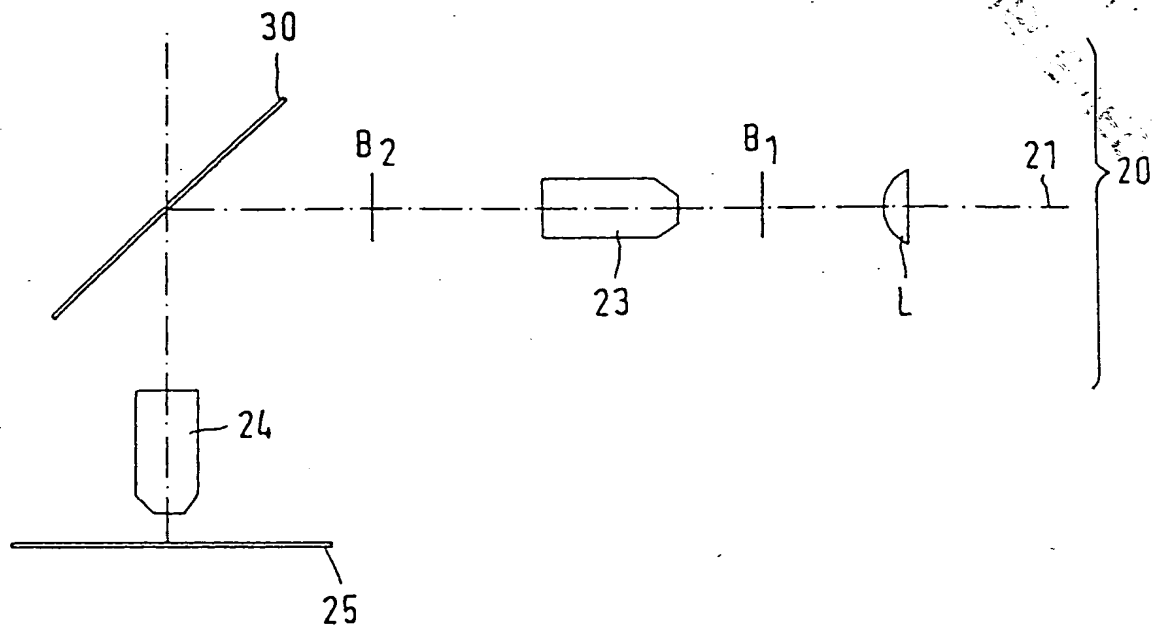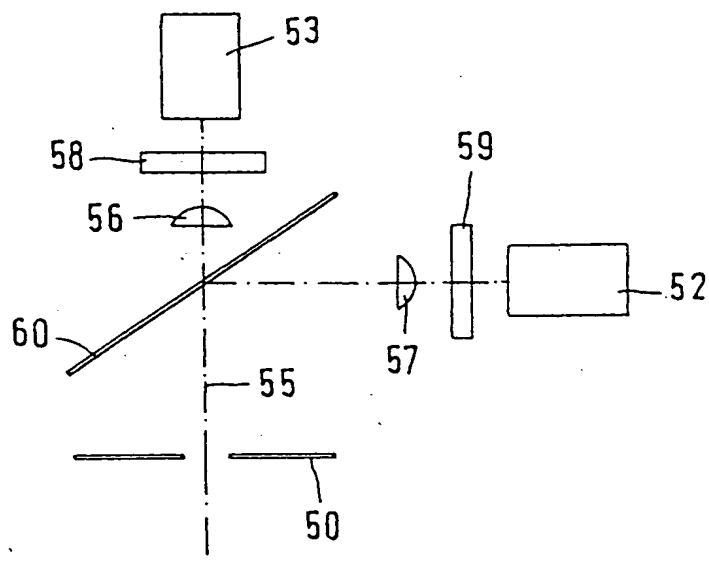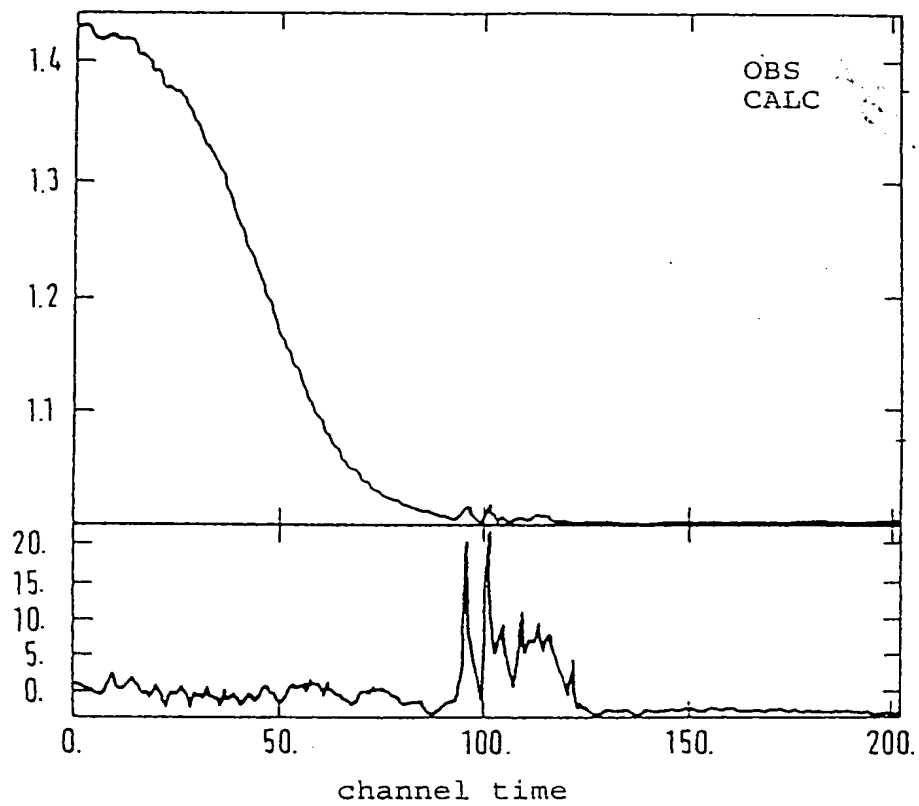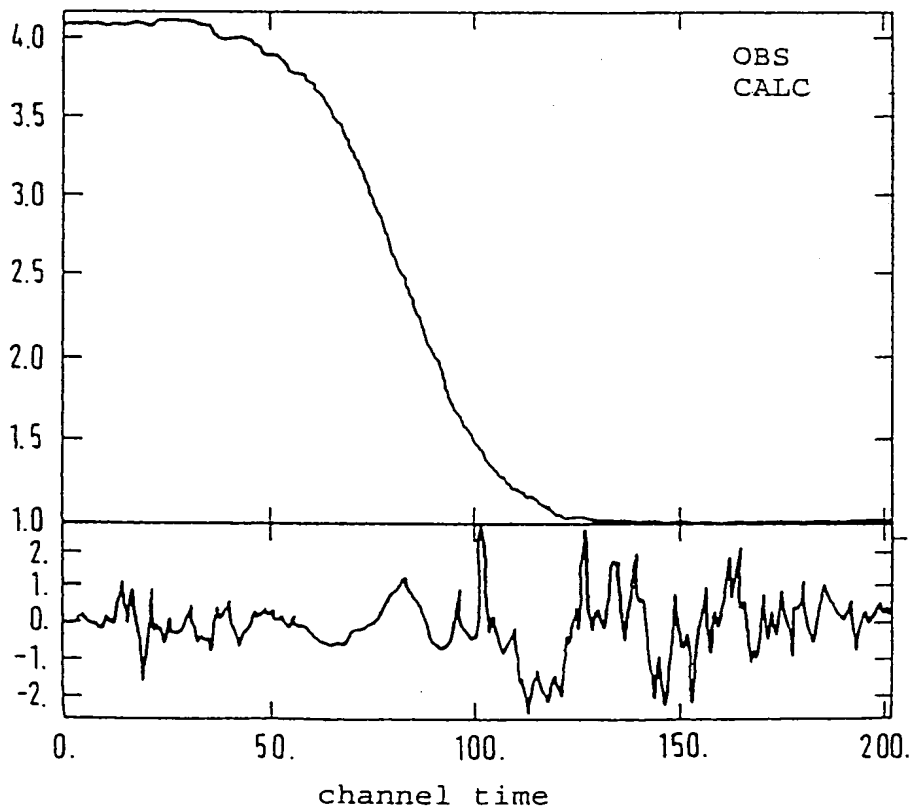
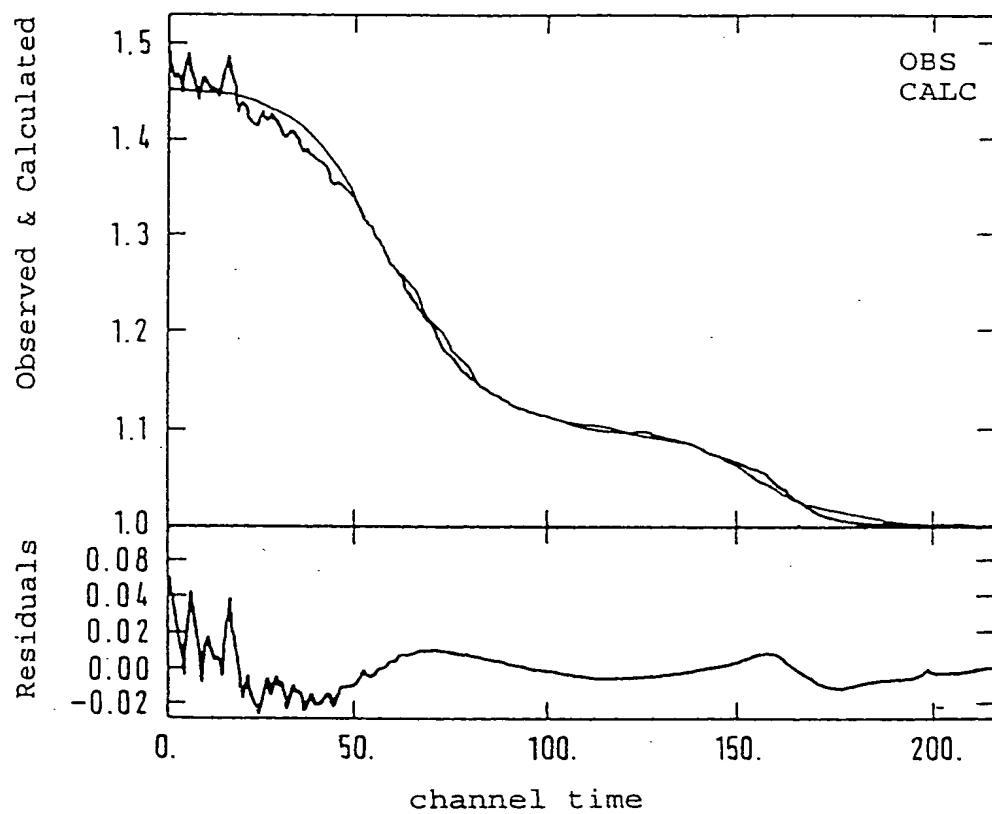# FIG. 18a
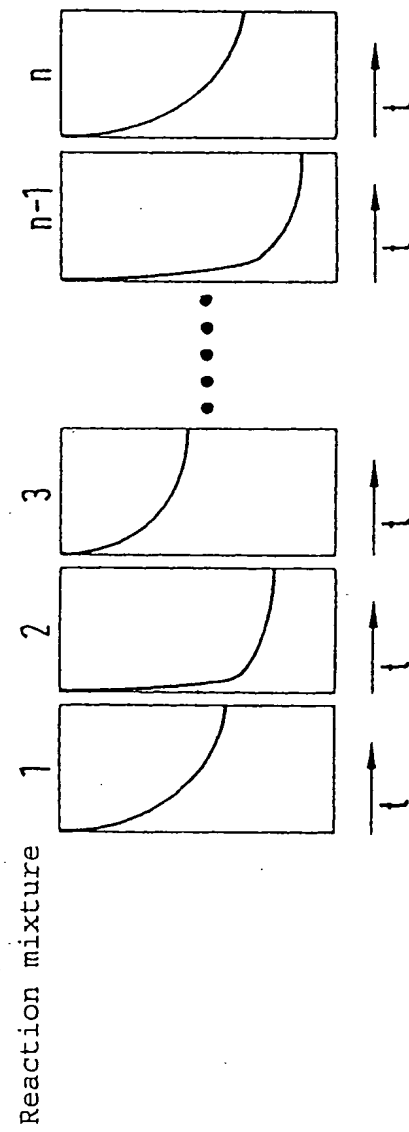

FIG. 18b

FIG. 18c

FIG. 19

Determination by FCS of the Dissociation Behavior
of Complexes in Experiments Performed in Parallel

Different Embodiments of the Electric Trap
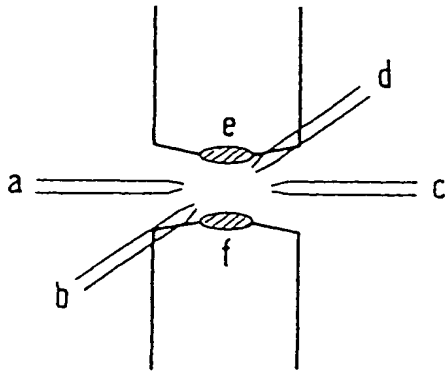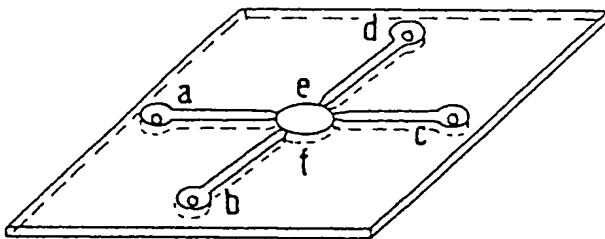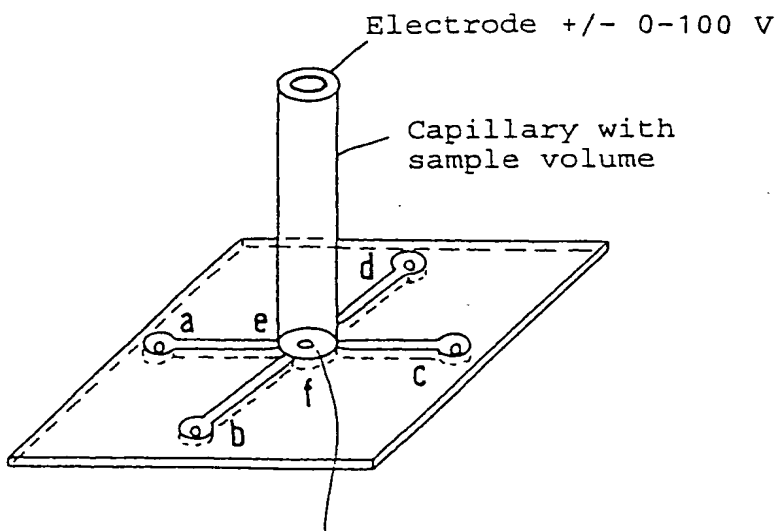According to the Invention

FIG. 20a

FIG. 20b

FIG. 20c

Electrode +/- 0-100 V

Capillary with
sample volume

Collecting electrode with earthing (potential 0 V) and Pinhole
for ions to pass into the quadrupolar field
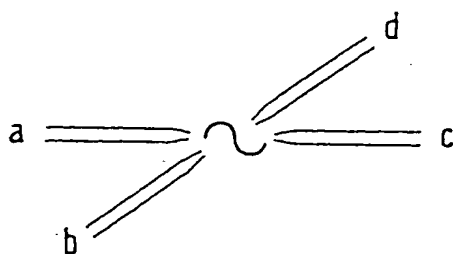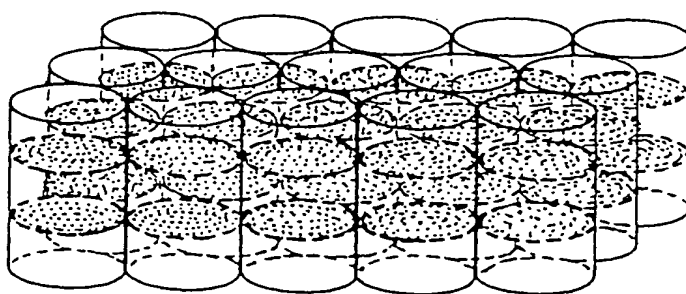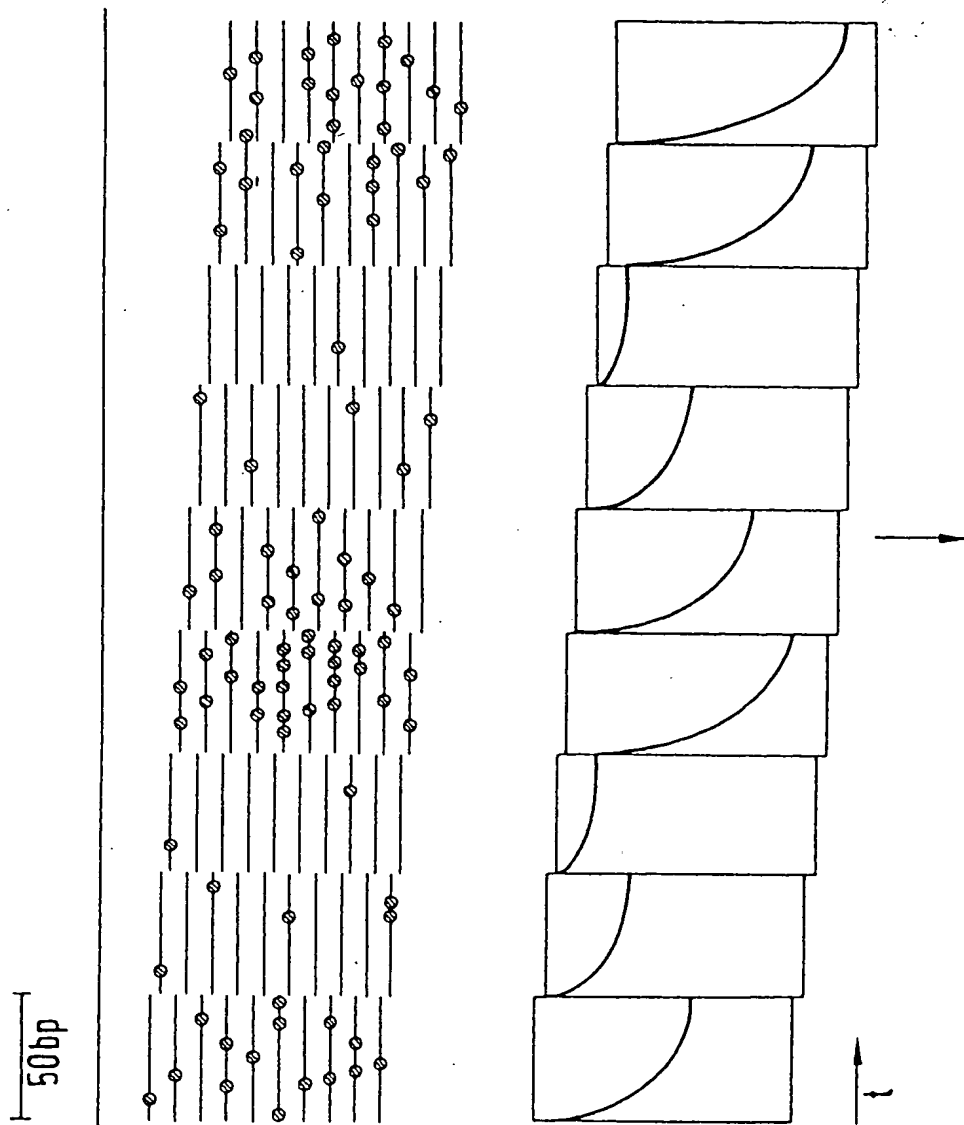
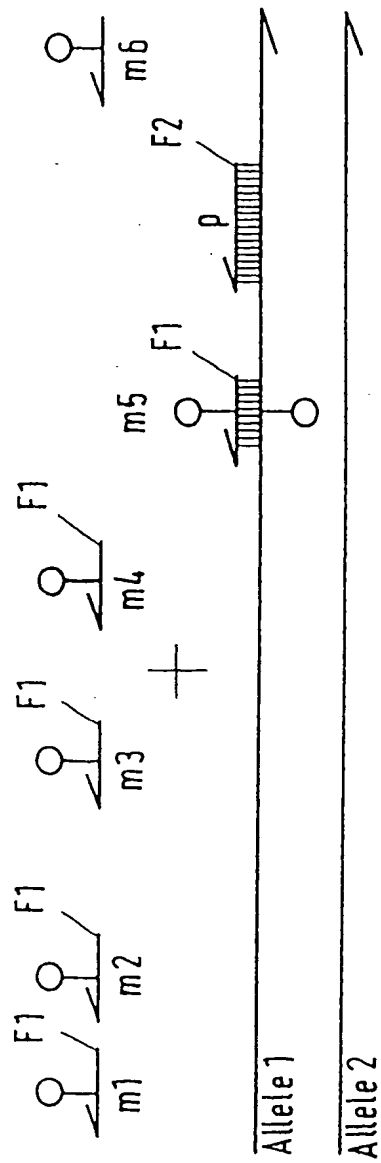Molecular Detection

FIG. 21a

FIG. 21b

FIG. 22

FIG. 23

Small Excitation Volumes (a) and
Small Measuring Volumes (b) and Small
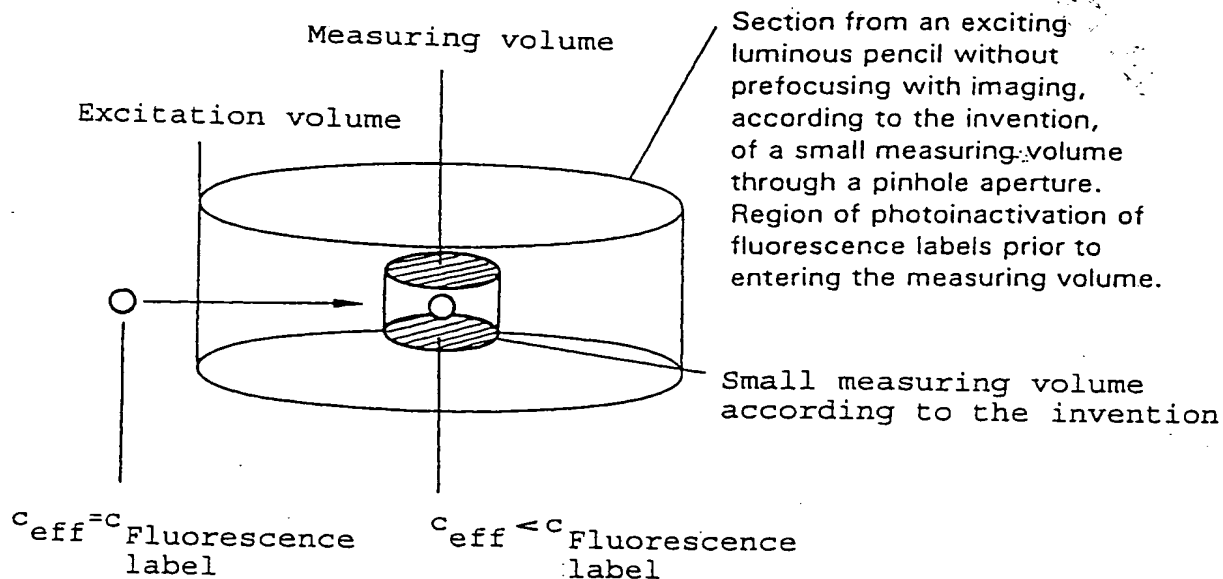Volumes with Parallel Measurements (c)

FIG. 24a

Measuring volume
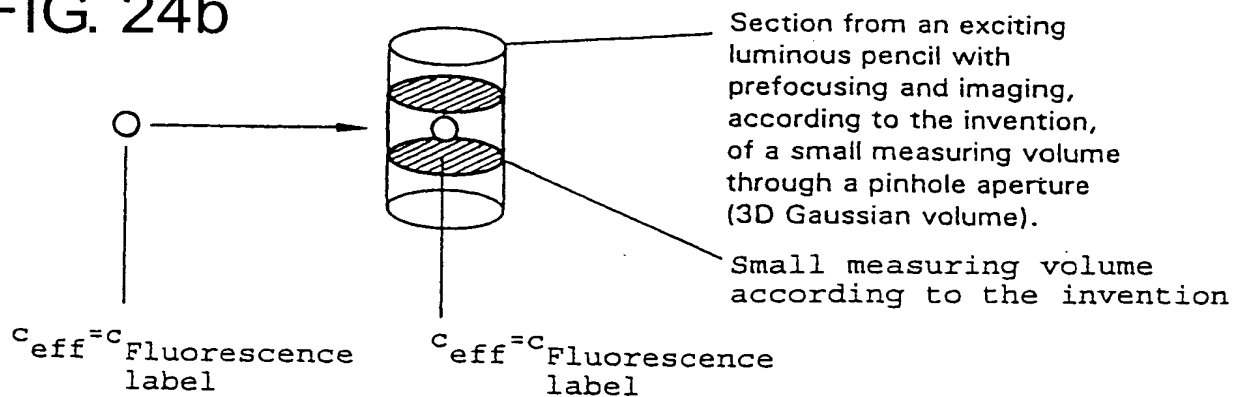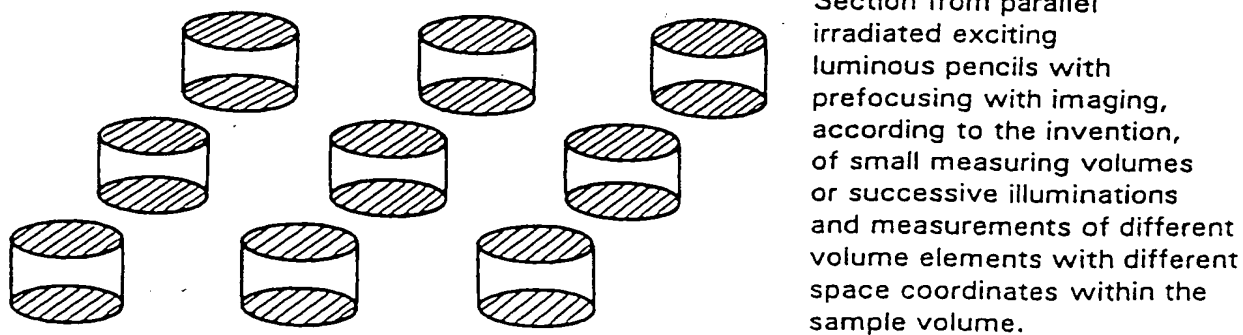
Excitation volume

Section from an exciting
luminous pencil without
prefocusing with imaging,
according to the invention,
of a small measuring volume
through a pinhole aperture.
Region of photoinactivation of
fluorescence labels prior to
entering the measuring volume.

Small measuring volume
according to the invention

$c_{eff} = c_{Fluorescence\ label}$

$c_{eff} < c_{Fluorescence\ label}$

FIG. 24b

Section from an exciting
luminous pencil with
prefocusing and imaging,
according to the invention,
of a small measuring volume
through a pinhole aperture
(3D Gaussian volume).

Small measuring volume
according to the invention

$c_{eff} = c_{Fluorescence\ label}$

$c_{eff} = c_{Fluorescence\ label}$

FIG. 24c

Section from parallel
irradiated exciting
luminous pencils with
prefocusing with imaging,
according to the invention,
of small measuring volumes
or successive illuminations
and measurements of different
volume elements with different
space coordinates within the
sample volume.

FIG. 25

FIG. 26a

FIG. 26b

FIG. 26c

OBS
CALC

channel time

FIG. 27

OBS
CALC

observed & calculated

channel time

FIG. 28a

Autocorrelation function (arbitrary units)

Time (Channel)

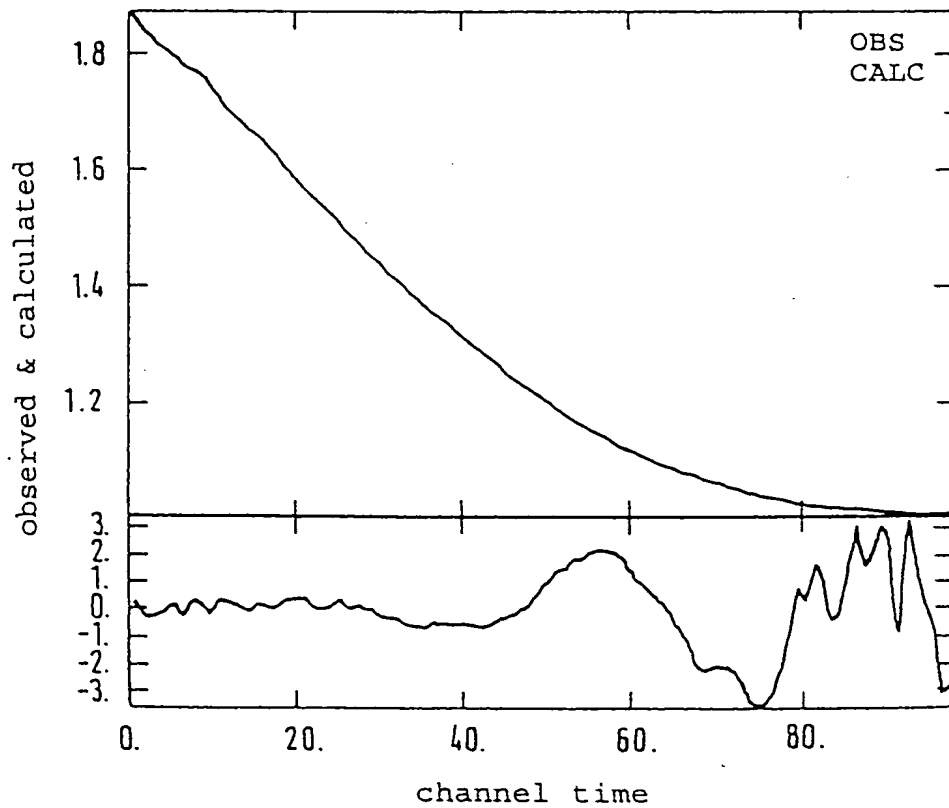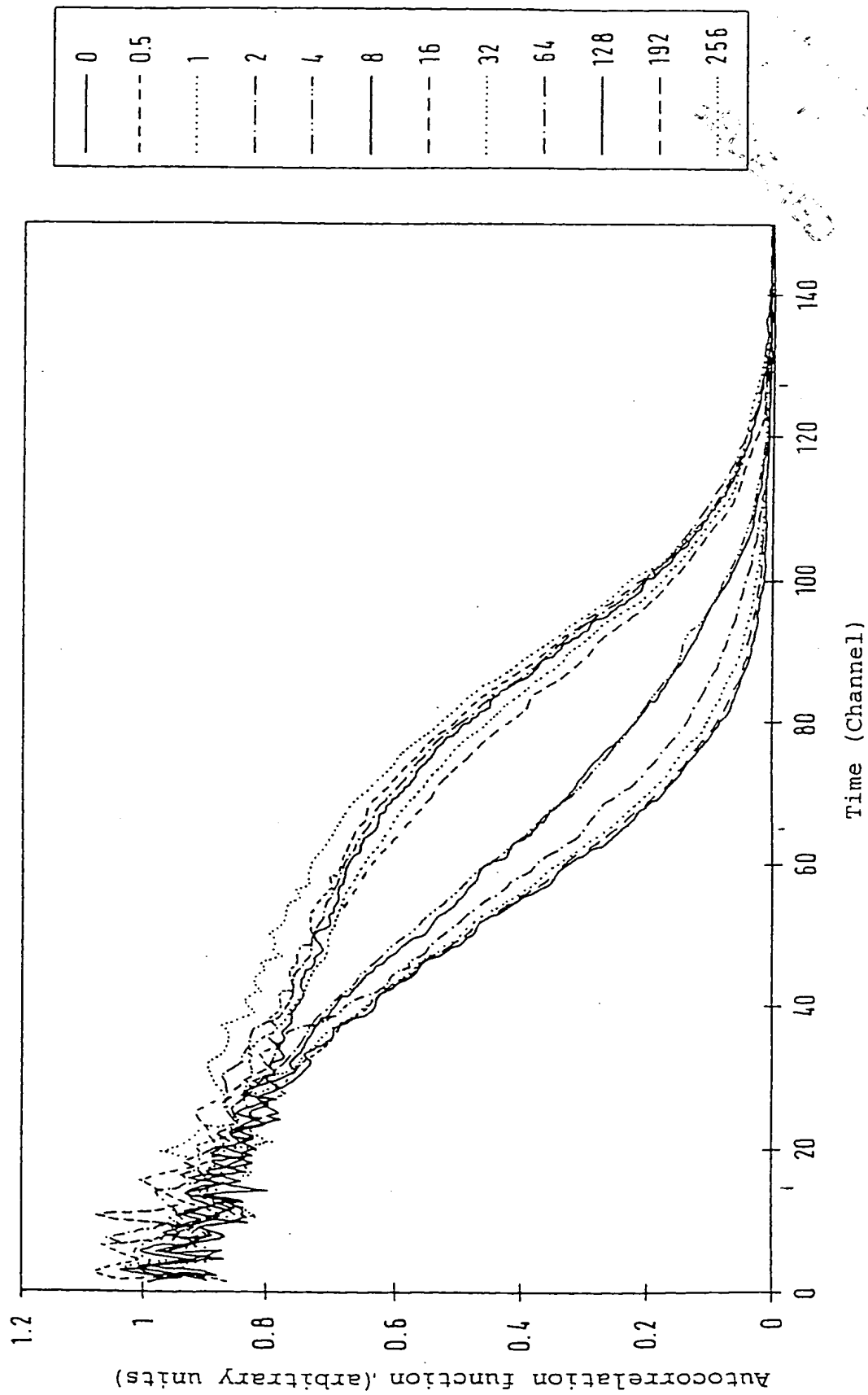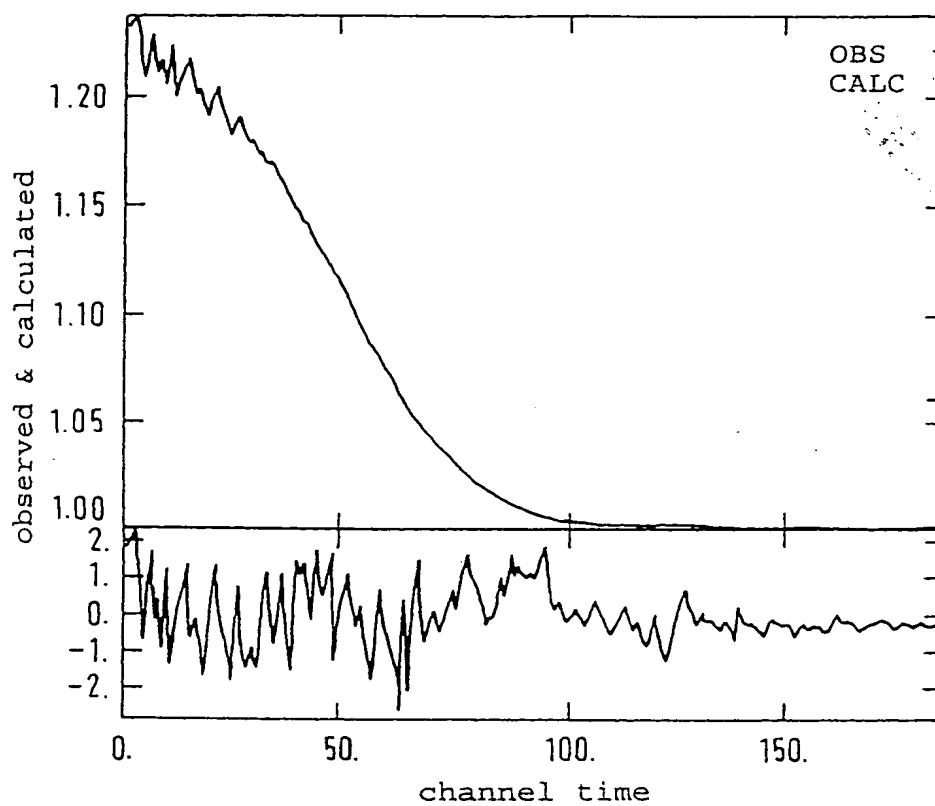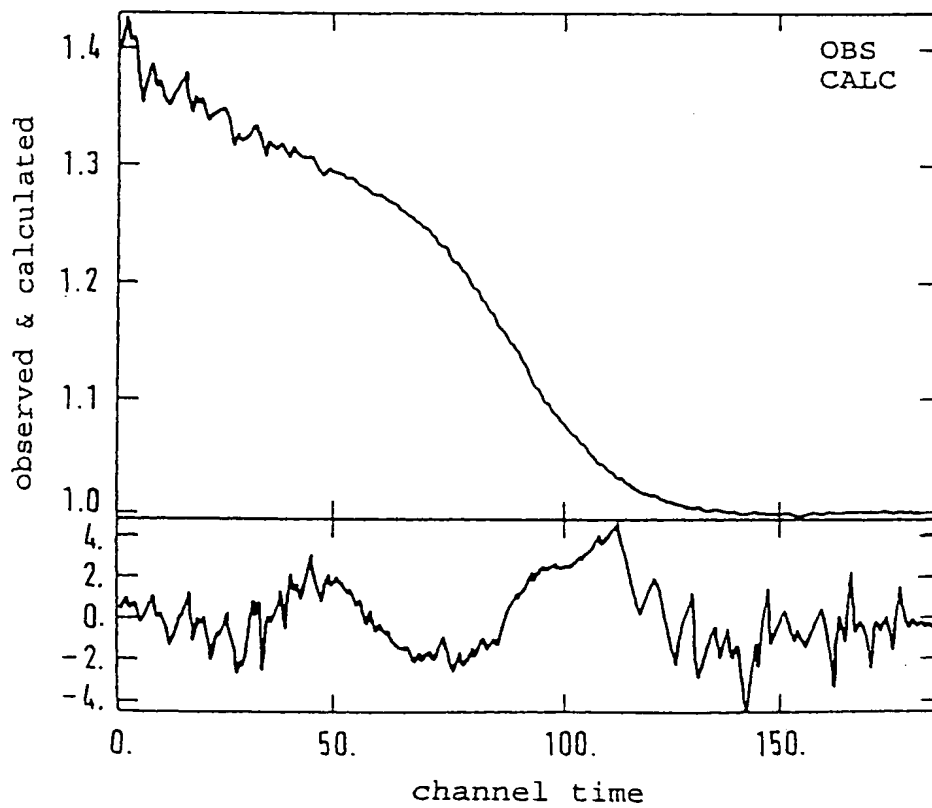|	
0	
0.5	
1	
2	
4	
8	
16	
32	
64	
128	
192	
256	
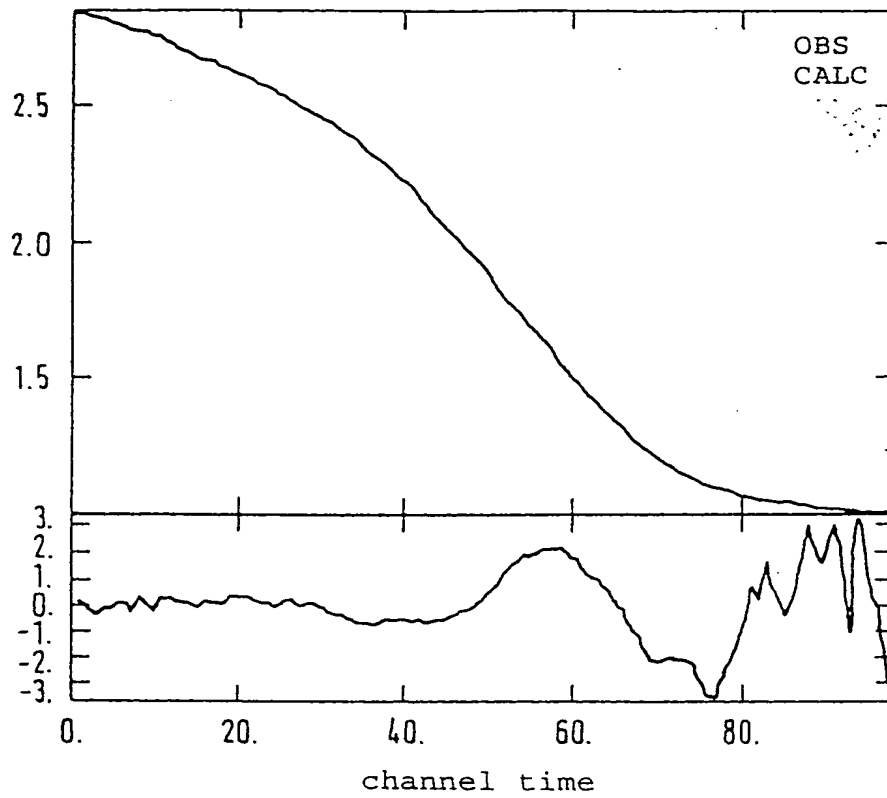
FIG. 28b

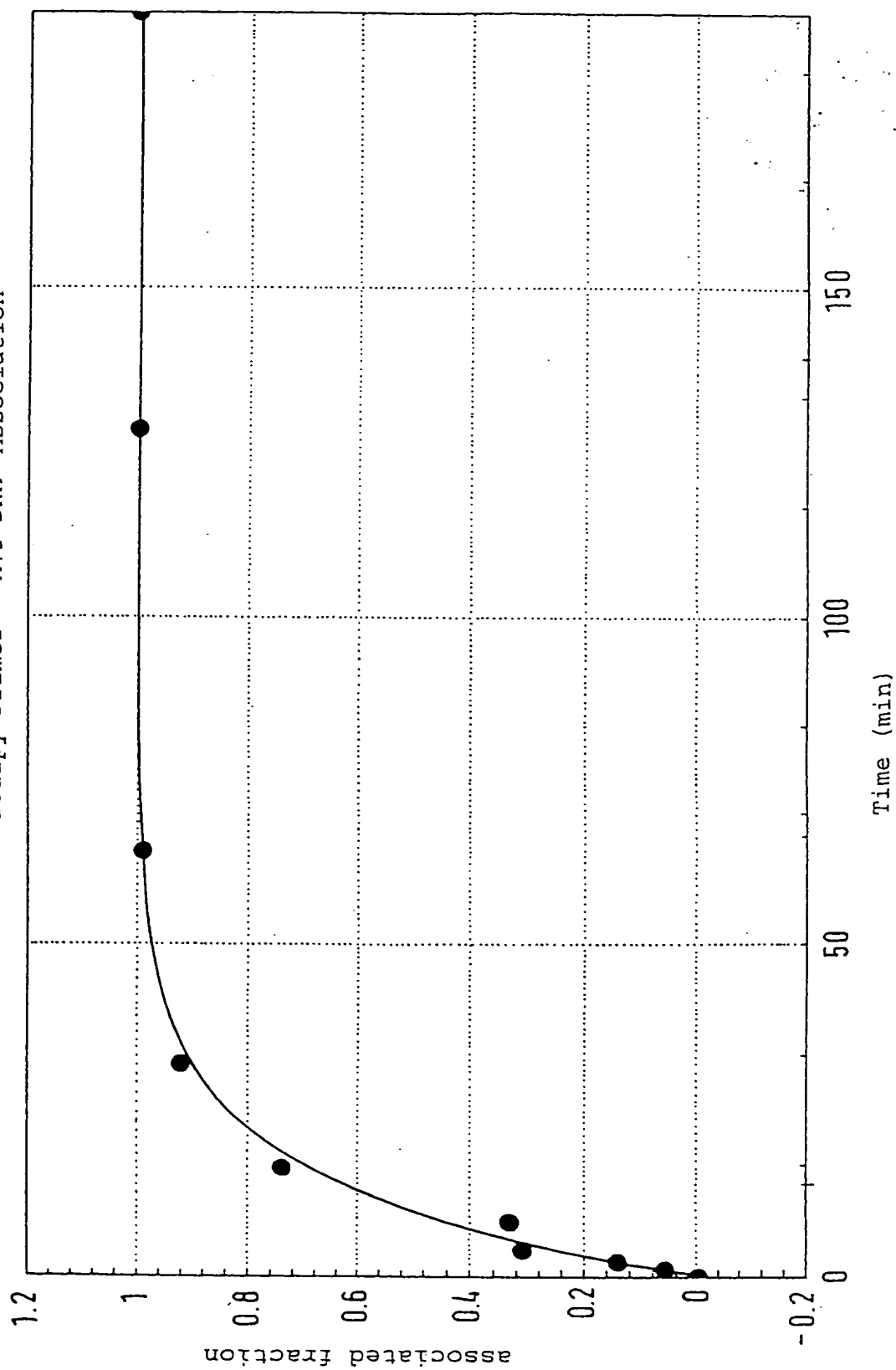# FIG. 28c

FIG. 29

Bodipy Primer - M13 DNA Association

FIG. 30

RDV10.DAT (Rho-dUTP with steel tips)

Amplitude: 2V, Frequency: 4 Hz

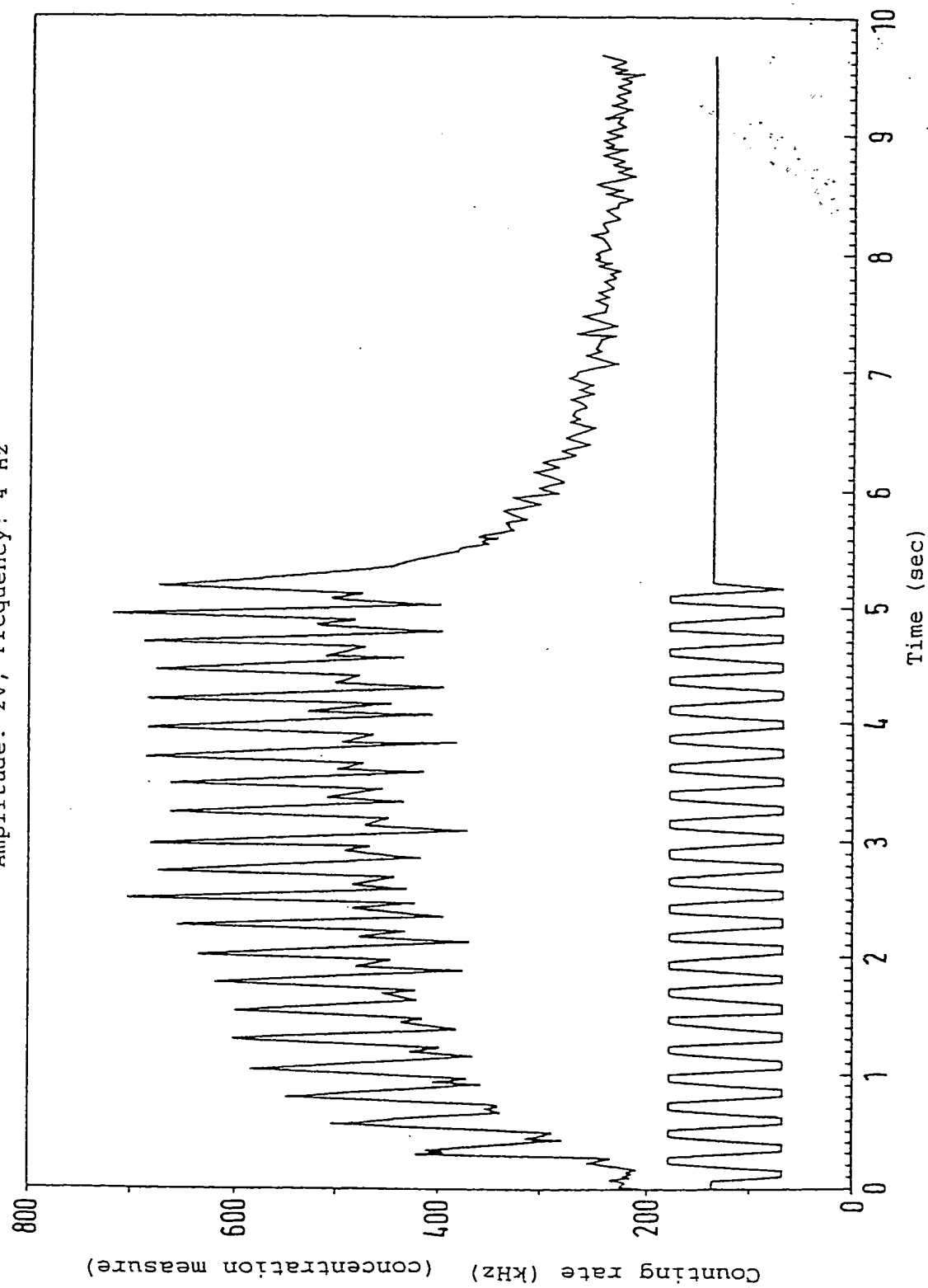# FIG. 31a

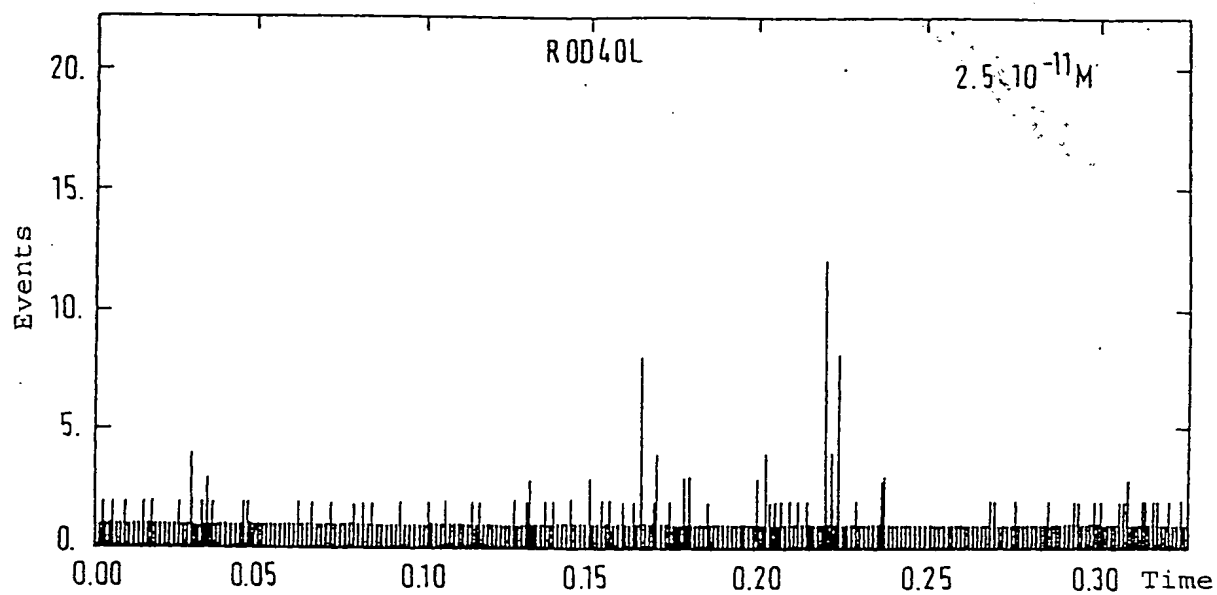Multichannel Detection of Rhodamine 6G (Single Molecules)

ROD40L 2.5×10^{-11} M

FIG. 31b

ROD40S $4 \cdot 10^{-10}$ M